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Synthesis of a Phthaloylglycine-Derived Strigol Analogue and Its Germination Stimulatory Activity toward Seeds of the Parasitic Weeds Striga hermonthica and Orobanche crenata

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The newly designed strigol analogue Nijmegen 1 (rac 7) was prepared in high overall yield starting from N-phthaloylglycine. This relatively simple analogue exhibits high bioactivity in the stimulation of germination of the parasitic weeds Striga hermonthica and Orobanche crenata. Nijmegen 1 was resolved in its enantiomers 7 and ent 7 by using the homochiral latent D-rings ent 11 and ent 12. The enantiomers 7 and ent 7 show significant differences in germination activity.

Keywords: Striga; Orobanche; germination; strigol analogue

INTRODUCTION

The devastating parasitic weeds Striga and Orobanche cause severe reductions in food crop yield of several graminaceous and leguminous crops in tropical and semitropical areas of the eastern hemisphere (Musselman, 1987; Parker and Riches, 1993). A strict requirement for the germination of the seeds of these parasitic weeds is exposure to a chemical substance that is usually present in the root exudate of a potential host plant (Press et al., 1990; Butler, 1995). An attractive control strategy for the eradication of infested fields is the concept of suicidal germination, i.e., introduction of a germination stimulating agent into the soil prior to sowing to induce germination of the parasitic seeds in the absence of a host plant (Eplee, 1975). The first known naturally occurring germination stimulant, (+)-strigol (1), was isolated from the root exudate of the false host cotton (Gossypium hirsutum L.) (Cook et al., 1966, 1972). Recently, (+)-strigol (1) was also identified in the root exudates of the Striga host plants maize (Zea mays L.) and proso millet (Panicum miliaceum L.) (Siame et al., 1993). In addition, some structurally closely related “strigolactones” (Butler, 1995) have been identified in the root exudates of other Striga hosts, viz. sorgolactone 2 (Hauck et al., 1992) and alectrol 3 (Müller et al., 1992).

However, strigolactones 1–3 (Figure 1) are not suitable for weed control purposes, because their structures are too complicated to allow synthesis in an economically feasible manner. Therefore, several studies aimed at synthetic analogues with a relatively simple structure but with high germination stimulatory activity (Johnson et al., 1976, 1981; Vail et al., 1990; Bergmann et al., 1993; Mangnus et al., 1992a; Zwanenburg et al., 1994). These studies mainly focused on the ABC-part of the strigolactones. In this part of the molecule a considerable structural variation is allowed to retain high biological activity. On the basis of these observations, a tentative molecular mechanism (Scheme 1), which accounts for the onset of the biochemical cascade leading to germination, has been proposed (Mangnus and Zwanenburg, 1992a). According to this mechanism the bioactiphore resides in the vinyl ether part of the D-ring.

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problem associated with the analogues 4–6 is the presence of two stereogenic centers in these molecules with the consequence that during their syntheses mixtures of diastereomers are obtained, which can be separated only by tedious chromatography. The present paper describes the synthesis of phthaloylglycine-derided strigol analogue rac 7, which we named Nijmegen 1, and the evaluation of the germination stimulatory activity toward seeds of Striga and Orobanche spp. This new germination stimulant contains only one chiral center, viz. in the D-ring. This compound was designed in such a manner that it contains the essential structural features for bioactivity (vide supra); that is, the essence of the molecular mechanism is not affected. Its planar phthalimido moiety is entirely divergent from that of the ABC-part of the strigolactones 1–3 and the GR analogues 4 and 5.

MATERIALS AND METHODS

Nomenclature. We have used the AUTONOM 1.0 program, provided by the Beilstein Institute and Springer-Verlag, Weinheim, Germany.

Syntheses. General Remarks. 1H NMR (100 MHz) spectra were recorded on a Bruker AC 100 spectrometer (MeSi as internal standard). 400 MHz 1H-NMR spectra were recorded on a Bruker AM-400 spectrometer (Me 4Si as internal standard), both from Bruker (Wissembourg, France). All coupling constants are given as J in hertz, unless indicated otherwise. For mass spectra a double-focusing VG7070E mass spectrometer from VG Analytical (Manchester, U.K.) was used. GLC was conducted with a Hewlett-Packard HP 5890 gas chromatograph, from Hewlett-Packard Nederland (Amstelveen, Netherlands), using a capillary cross-linked methyl silicone gum column of 25 m length and 0.32 mm i.d., with a 0.17 μm film thickness and nitrogen (2 mL/min, 0.5 atm) as carrier gas. Melting points were measured with a Reichert Thermopan (Austria) microscope and are uncorrected. Elemental analyses were performed at the Department of Microanalysis of this laboratory.

Solvents were dried using the following methods: Dichloromethane was distilled from P2O5. Diethyl ether was distilled from NaH. Hexane was distilled from CaH2. Tetrahydrofuran was distilled from lithium aluminum hydride just before use. All other solvents were of analytical grade. Thin layer chromatography (TLC) was carried out on Merck (Darmstadt, Germany) precoated silica gel 60 F 254 plates (0.25 mm) using the eluents indicated. Spots were visualized with UV or using a molybdate spray. “Flash” chromatography was carried out at a pressure of ca. 1.5 bar, using Merck Kieselgel 60H. Column chromatography at atmospheric pressure was carried out using Merck Kieselgel 60.

The synthesis of chlorolactones 11 and ent 11 was reported previously (Thuring et al., 1995).

Methyl 2-(1,3-Dioxo-1,3-dihydroisindol-2-yl)-3-(4-methyl-5-oxo-2,5-dihydrofuran-2(R)-yloxy)acrylate (rac 7). Potassium tert-butoxide (372 mg, 3.32 mmol) was added to a cooled (0°C) and stirred solution of Sheehan aldehyde 9 (745 mg, 3.02 mmol) in DMF (10 mL) at room temperature under nitrogen. Then chlorofuranone 10 (480 mg, 3.62 mmol) in DMF (3 mL) was gradually added. The mixture was stirred at room temperature over a weekend. DMF was removed in vacuo, and the residue was dissolved in a mixture of water and ethyl acetate. The organic layer was separated, and the aqueous phase was extracted with ethyl acetate (two times). The combined organic layers were washed with water (two times), dried (MgSO4), and concentrated in vacuo. The oily residue was triturated with diisopropyl ether. Almost pure rac 7 (660 mg, 64%) was isolated as a white solid by filtration and washing with diisopropyl ether. An analytical sample was obtained by recrystallization from 2-propanol. mp 151–152°C. 1H NMR (CDCl3, 400 MHz) δ 1.97 (br s, 3H, CH3), 3.78 (s, 3H, OCH3), 6.17 (br s, 1H, OCHO), 6.90 (br s, 1H, Ar), 7.76 (m, 2H, 2 arom H), 7.90 (m, 3H, 2 arom H + –CHO); MS [M+ 1/m2, rel intensity (%)] 343 ([M]+, 2.7), 246 ([C12H10O4]+), 100, 97 ([C12H8O]+), 59.3. Anal. Calc. for C12H10NO5: C, 59.18; H, 3.82; N, 4.08. Found: C, 59.10; H, 3.85; N, 4.00.

Methyl 2-(1,3-Dioxo-1,3-dihydroisindol-2-yl)-3-(6-methyl-5-oxo-2,5-dihydrofuran-2(R)-yloxy)acrylate (rac 12). Potassium tert-butoxide (149 mg, 1.33 mmol) was added to a stirred solution of Sheehan aldehyde 9 (302 mg, 1.22 mmol) in DMF (10 mL) at room temperature under nitrogen. Then chlorolactone 11 (265 mg, 1.33 mmol) in DMF (3 mL) was gradually added. The mixture was stirred at 55°C for 7 days and then quenched with acetic acid (0.5 mL). DMF was removed in vacuo, and the residue was dissolved in a mixture of water and ethyl acetate. The organic layer was separated, and the aqueous phase was extracted with ethyl acetate (two times). The combined organic layers were washed with water (two times), dried (MgSO4), and concentrated in vacuo. The crude product was purified by flash chromatography (SiO2, hexane/ethyl acetate 2:1) to give 12 (143 mg, 29%) as a yellowish solid. An analytical sample was obtained by recrystallization from diisopropyl ether/ethyl acetate. mp 179.5–181°C. [α]D2 – 22° (c 0.2, CHCl3); 1H NMR (CDCl3, 400 MHz) δ 1.46 (s, 3H, CH3), 1.65 (m, 2H, H2d), 2.62 (dd, 1H, J = 4.2 Hz, J = 11 Hz), 2.85 (m, 1H, H2b), 3.16 (m, 1H, H1b), 3.77 (s, 1H, OCH3), 6.16 (d, 1H, J = 11 Hz), 6.17 (s, 1H, O), 6.28 (m, 1H, H2a), 7.77 (m, 2H, Ar H), 7.85 (s, 1H, –CHO), 7.91 (m, 2H, Ar H); MS [M+ 1/m2, rel intensity (%)] 409 ([M]+, 0.6), 344 ([C12H10NO4]+), 247 ([C12H9NO3]+), 43.6, 163 ([C12H9O2]+), 78.2, 97 ([C12H8O]+), 100, 66 ([C11H8]+), 135. Anal. Calc. for C12H10NO5: C, 64.54; H, 4.68; N, 3.43. Found: C, 64.52; H, 4.63; N, 3.48.

Methyl 2-(1,3-Dioxo-1,3-dihydroisindol-2-yl)-3-[4-methyl-5-oxo-2,5-dihydrofuran-2(R)-yloxy]acrylate (ent 12). This compound was prepared in the same way as described for 12, starting from Sheehan aldehyde 9 (641 mg, 2.64 mmol) in DMF (3 mL), and chlorolactone ent 11 (530 mg, 2.67 mmol). Yield: 286 mg, 29% of ent 12 as yellowish solid. Recrystallization from diisopropyl ether/ethyl acetate afforded analytically pure ent 12. mp 179.5–181°C; [α]D2 + 23° (c 0.2, CHCl3). Anal. Calc. for C12H10NO5: C, 64.54; H, 4.68; N, 3.43. Found: C, 64.50; H, 4.64; N, 3.47. 1H-NMR and mass data were the same as for compound 12.

Methyl 2-(1,3-Dioxo-1,3-dihydroisindol-2-yl)-3-(4-[(5-methyl-5-oxo-2,5-dihydrofuran-2(R)-yloxy)acryloyl]acetate (7), Cycloadduct 12. Methyl 2-(1,3-Dioxo-1,3-dihydroisindol-2-yl)-3-[4-methyl-5-oxo-2,5-dihydrofuran-2(R)-yloxy]acrylate (7). Cycloadduct 12 (159 mg, 0.39 mmol) was dissolved in o-dichlorobenzene (40 mL) and heated at 180°C for 7 h. The solvent was removed in vacuo. The residue was purified by flash chromatography (SiO2, hexane/ethyl acetate 1:1) to give 7 (53 mg, 40%) as a colorless oil, which failed to crystallize. [α]D2 + 124° (c 0.15, CHCl3). 1H-NMR and mass data were the same as for compound rac 7.
Synthesis and Bioactivity of a Glycine-Derived Strigol Analogue

Scheme 2. Preparation of rac 7

![Scheme 2](image)

Methyl 2-[(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-[4-methyl-5-oxo-2,5-dihydrofuran-2(S)-yloxy]acrylate (ent 7) was prepared in the same way as described for 7, starting from ent 12 (230 mg, 0.56 mmol). Yield: 43 mg, 31% of which failed to crystallize.

Biological Activity. Seeds. Seeds of Striga hermonthica (Del.) Benth [from Sorghum bicolor (L.) Moench] and Orobanche crenata Forsk. (from Vicia faba L.) were harvested in Sudan in 1988 and in Egypt in 1991, respectively, and were stored in the dark at room temperature until use in germination tests.

Preparation of Test Solutions. A compound to be tested was weighed out very accurately to the amount of 10 mg, dissolved in 10 mL of acetone p.a., and diluted with demineralized water to 100 mL. Aliquots of this stock solution were further diluted with water to obtain test solutions containing 2, 1, 0.1, and 0.01 mg/L test compound and 0.2, 0.1, 0.01, and 0.001% (v/v) acetone, respectively.

Bioassays. For surface sterilization seeds of S. hermonthica and O. crenata were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) for 5 min with agitation. The seeds were then thoroughly rinsed with water and dried overnight.

For conditioning the sterilized seeds were spread on glass fiber filter paper disks (8-mm diameter; approximately 30–70 seeds per disk) in Petri dishes, wetted with water, and stored in the dark for 14 days at 20 °C for Orobanche seeds and at 30 °C for Striga seeds. Then the conditioning water was removed and replaced by 100 µL of test solution per disk. After incubation for 24 h (Striga) and 5 days (Orobanche) in the dark at indicated temperatures, the germination percentage was determined using seeds of S. hermonthica as the Z-isomer standard.

In each test series aqueous solutions with 0.2, 0.1, 0.01, and 0.0001% (v/v) acetone were used as negative controls. Test solutions of the stimulant GR24 (as a 1:1 diastereomeric mixture containing 1, 0.1, and 0.001 mg/L) were used as positive controls. All tests were performed in duplicate, and in each test the germination percentages were determined on 12 disks per treatment.

For full details of the bioassay, see Mangnus et al. (1992c).

RESULTS AND DISCUSSION

The key step in the synthesis of rac 7 involves coupling of aldehyde 9 with 5-chloro-3-methyl-2(5H)-furanone (10). This aldehyde 9 was prepared by condensation of methyl N-phthaloylglycinate (8) with methyformate using metallic sodium (Scheme 2).

This procedure, which closely resembles that described by Schutz (1978), is superior to that originally reported by Sheehan and Johnson (1954). It should be noted that 9 is a stable, crystalline compound, which can be stored for several years. The coupling reaction with butenolide 10 (Scheme 2) proceeded in high yield, and purification was readily accomplished by recrystallization. It is important to note that only one geometrical isomer was obtained. The correct geometrical structure could not be deduced unambiguously by spectroscopic means, and therefore an X-ray diffraction analysis was undertaken (Beurskens et al., 1994). The structure of 7 is depicted in Figure 2, showing that the Z-isomer was obtained.

Next, the preparation of the individual enantiomers of 7 was attempted, using enantiomerically pure tricyclic chloroacetonitrile 11 and ent 11 as the D-ring precursors (Scheme 3). The stereoselective synthesis of 11 and ent 11 and their use in the preparation of the single isomers of strigol analogues has been reported recently (Thuring et al., 1995).

The coupling reactions of Sheehan aldehyde 9 with 11 and ent 11 did not proceed as smoothly as was observed for the corresponding GR7 analogues (Thuring et al., 1995). As a result of the relatively poor nucleophilicity of the enolate anion derived from 9, a higher reaction temperature was required, which caused concomitant decomposition of 11 and ent 11. The cycloreversion of 12 and ent 12 was performed in o-dichlorobenzene at 180 °C to give 7 and ent 7, respectively, in moderate yields. The ee values of both enantiomers were > 98%, as was determined by 1H-NMR analysis using the chiral shift reagent Eu(hfc)3.

Biological Evaluation. The germination stimulatory activity of Nijmegen 1 (rac 7) was assayed using seeds of S. hermonthica and O. crenata spp. In each bioassay, GR24 was included as a positive control. The procedure enables a comparison between results obtained in different test series. This is important, since the response of seeds of parasitic weeds, especially S. hermonthica, varies considerably from test to test. In addition, the activities of enantiomers 7 and ent 7 were determined using seeds of O. crenata spp. The results
are collected in Tables 1 and 2. It was beyond the aim of this study to establish complete dose–response curves, implying that the data obtained allow only an interpretation in a qualitative sense.

The data in Table 1 (S. hermonthica spp.) reveal that rac Nijmegen 1 exhibits considerable activity at the higher concentration of 1 ppm, whereas it is practically inactive at a concentration of 0.01 ppm. Similarly, in the stimulation of O. crenata spp. seeds, rac 7 has shown a bioactivity comparable to that of GR24 at higher concentrations (entry 1, Table 2). Comparison of the germination percentages exerted by enantiopure 7 and ent 7 (entries 2 and 3, Table 2) reveals that the former is considerably more active. Thus, the absolutestereochemistry at C-2′ in the D-ring should be the R-configuration to germinate a maximum number of seeds. This configuration is the same as in natural (+)-strigol. This result is in agreement with previous conclusions from comparative studies of the bioactivity of all stereoisomers of GR7 (Mangnus and Zwanenburg, 1992b) and of some stereoisomers of strigol (Bergmann et al., 1993), namely, that the most active stereoisomer has the R-configuration at C-2′ in the D-ring.

From the results presented above, it may be concluded that phthaloylglycine-derived strigol analogue rac 7 is a potent germination stimulant of seeds of S. hermonthica and O. crenata spp. Moreover, optically active 7 with the “natural” configuration in the D-ring has a stimulatory activity comparable to that of GR24 for O. crenata. The charm of this particular stimulant is the fact that its racemic preparation is very simple and that it can be carried out without any chromatographic separation, which makes it an attractive compound for large-scale preparations and accordingly for use in the suicidal approach in the weed pest control. Moreover, the achiral “ABC”-part in rac 7 enables a rapid evaluation of the structural variation in the D-ring on the stimulatory activity. Research in this direction is in progress.

It should be noted that our newly developed asymmetric route allows for the first time the synthesis of a strigol analogue, which is only chiral at the D-ring. The ease of preparation and the high bioactivity of this new germination stimulant warrant further studies to evaluate its activity and stability under soil conditions. Activities in this direction are in progress.

From a mechanistic point of view we can conclude that a possible interaction of the ABC-fragment with a receptor site is sterically and electronically not highly demanding.

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LITERATURE CITED


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